

# Locally administered low-dose alendronate increases bone mineral density during distraction osteogenesis in a rabbit model

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**We investigated the effect of locally administered bisphosphonate on distraction osteogenesis in a rabbit model and evaluated its systemic effect. An osteotomy on the right tibia followed by distraction for four weeks was performed on 47 immature rabbits. They were divided into seven equal groups, with each group receiving a different treatment regime. Saline and three types of dosage of alendronate (low, 0.75 µg/kg; mid, 7.5 µg/kg and high 75 µg/kg) were given by systemic injection in four groups, and saline and two dosages (low and mild) were delivered by local injection to the distraction gap in the remaining three groups. The injections were performed five times weekly during the period of distraction.**

**After nine weeks the animals were killed and image analysis and mechanical testing were performed on the distracted right tibiae and the left tibiae which served as a control group. The local low-dose alendronate group showed a mean increase in bone mineral density of 124.3 mg/cm<sup>3</sup> over the local saline group (analysis of variance,  $p < 0.05$ ) without any adverse effect on the left control tibiae.**

**The findings indicate that the administration of local low-dose alendronate could be an effective pharmacological means of improving bone formation in distraction osteogenesis.**

Distraction osteogenesis is a widely practised orthopaedic technique used for reconstruction in congenital disorders,<sup>1</sup> bone restoration after traumatic defects<sup>2</sup> and for neoplastic conditions,<sup>3</sup> but the method has the disadvantage of a prolonged healing time. The healing index for limb lengthening is calculated by dividing the period with the fixator in place by the length of bone gained. It is reported to range between 28 days/cm and 36 days/cm.<sup>4,5</sup> There have been several studies which have investigated methods of accelerating this.<sup>6,7</sup>

Bisphosphonates are stable pyrophosphate analogues which are distributed mainly to sites of exposed hydroxyapatite where they inhibit bone resorption.<sup>8</sup> They are used in the treatment of osteoporosis, osteolytic bone disease and hypercalcaemia because of malignancy.<sup>9-12</sup> In animal experiments the administration of the nitrogen-containing bisphosphonate (N-BP) by intravenous injection has been shown to increase the strength, bone mineral content and bone mineral density (BMD) during distraction osteogenesis.<sup>13,14</sup> However, in mice high-dose N-BP caused adverse effects, such as osteonecrosis of the jaw and inhibition of the growth of long bones by alteration of the growth plate as a result of

reduced resorption at the chondro-osseous junction.<sup>15,16</sup>

Bisphosphonates have a high affinity for bone mineral and local application is feasible.<sup>17,18</sup> Low-dose N-BP administered locally is expected to increase the bone formation in distraction osteogenesis by achieving a high local concentration, which should inhibit bone absorption by osteoclasts more effectively than that obtained by systemic administration.<sup>19</sup> Although there have been few reports on the local administration of bisphosphonates in conjunction with distraction osteogenesis, Amanat et al<sup>20</sup> found that local application of pamidronate increased the bone mineral content in a model of rat fracture. Before considering the local application of bisphosphonates in clinical use, the systemic effect of the application should be determined, since little is known about the dynamic state of locally-administered bisphosphonate.

Using mechanical and unique analysis we have investigated whether locally-injected low-dose N-BP would enhance bone formation during distraction osteogenesis. Additionally, we evaluated the systemic effect of local treatment with N-BP. A previous study by Yaffe et al<sup>17</sup> on the local delivery of alendronate to the man-

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**Table I.** Details of the experimental design

| Treatment group | Number of rabbits | Injection site            | Dose of alendronate (µg/kg) | Dose of saline (ml) |
|-----------------|-------------------|---------------------------|-----------------------------|---------------------|
| Systemic-saline | 7                 | Back, subcutaneous tissue | Nil                         | 0.2                 |
| Systemic-low    | 7                 | Back, subcutaneous tissue | 0.75                        | 0.2                 |
| Systemic-mid    | 7                 | Back, subcutaneous tissue | 7.5                         | 0.2                 |
| Systemic-high   | 5                 | Back, subcutaneous tissue | 75                          | 0.2                 |
| Local-saline    | 7                 | Distraction gap           | Nil                         | 0.2                 |
| Local-low       | 7                 | Distraction gap           | 0.75                        | 0.2                 |
| Local-mid       | 7                 | Distraction gap           | 7.5                         | 0.2                 |

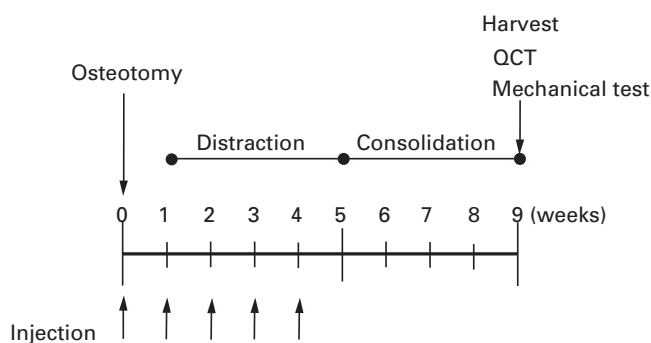


Fig. 1

Diagram of the experimental design. All the injections were performed immediately post-operatively and at one, two, three and four weeks after the operation (OCT, quantitative CT).

dible showed that the amount of alendronate detectable in the opposite side of the jaw when used as a control may reach up to one-third of the amount absorbed by the treated side.

**Materials and Methods**

**Bone lengthening and administration of alendronate.** We used 47 male Japanese white rabbits aged ten weeks and weighing between 1.9 kg and 2.2 kg. Our study followed the Guidelines<sup>18</sup> for Animal Experimentation of Hiroasaki University, Japan.

The animals underwent a standardised open transverse osteotomy of their right tibial diaphysis. They were anaesthetised by intravenous injection of 50 mg/kg of pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, Illinois). An incision 4 cm long was made over the medial aspect of the right tibia and a Pennig-mini lengthener (Orthofix, McKinney, Texas) was secured by four 2.0 mm Kirschner wires. After the fixator had been applied, an osteotomy was performed on the tibia 5 mm distal to the tibiofibular junction using a 0.57 mm diameter thread saw (T-saw; Stryker, Kalamazoo, Michigan) under saline irrigation. Distraction was initiated after a latency period of seven days and continued at a rate of 0.75 mm per day for four weeks producing a total of 21 mm of distraction. The animals were allowed to eat and drink freely, their food consisting of standard rabbit pellet chow.

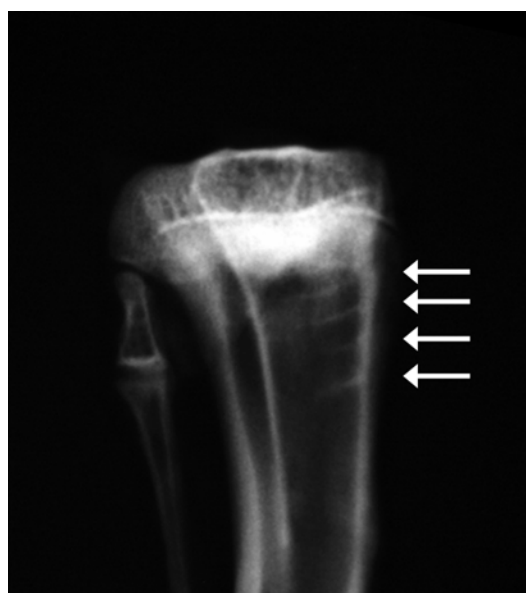


Fig. 2

An anteroposterior radiograph of the proximal tibia showing the presence of radiodense lines (white arrows).

A solution of alendronate (Merck, Rahway, New Jersey) was prepared in saline at a concentration of 0.05 mg/ml for the low-dose group, 0.5 mg/ml for the mid-dose group, and 5.0 mg/ml for the high-dose group. The high dose was prepared to provide a dose similar to that used in experiments on fracture healing stimulated by systemic injection.<sup>21</sup> The mid and low doses were prepared as 1/10 and 1/100 of the high dose, respectively. The rabbits were assigned to one of seven groups as follows: 1) the systemic-saline group, receiving 0.2 ml of saline; 2) the systemic low group receiving 0.75 µg/kg of alendronate; 3) the systemic-mid group receiving 7.5 µg/kg of alendronate; 4) the systemic-high group receiving 75 µg/kg of alendronate; 5) the local-saline group receiving 0.2 ml of saline; 6) the local low group receiving 0.75 µg/kg of alendronate, and 7) the local-mid group receiving 7.5 µg/kg of alendronate (Table I). The systemic groups received a subcutaneous injection in the back and the local groups a local injection into the distribution gap. All the injections were performed aseptically with a 23-gauge needle immediately post-operatively and at one, two, three and four weeks after operation (Fig. 1). After a

**Table II.** Data from quantitative CT and three-point bending test for all groups

|                                        | Systemic-saline             | Systemic-low                | Systemic-mid                | Systemic-high               | Local-saline                | Local-low                  | Local-mid                 |
|----------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|---------------------------|
| <b>Right distracted tibiae*</b>        |                             |                             |                             |                             |                             |                            |                           |
| Mean BMC (mg) (range)                  | 51.2<br>(40.3 to 69.3)      | 52.2<br>(36.1 to 76.2)      | 40.8<br>(18.4 to 56.3)      | 80.2<br>(56.3 to 113.3)     | 45.1<br>(21.2 to 65.4)      | 55.6<br>(35.2 to 87.9)     | 59.9<br>(31.8 to 72.1)    |
| Mean BMD (mg/cm <sup>3</sup> ) (range) | 543.6<br>(481.1 to 643.0)   | 566.2<br>(430.7 to 639.0)   | 525.8<br>(345.2 to 597.2)   | 653.1<br>(551.3 to 735.6)   | 456.3<br>(330.0 to 556.9)   | 580.6<br>(510.6 to 625.8)  | 560.6<br>(435.4 to 638.8) |
| Mean CSA (mm <sup>2</sup> ) (range)    | 94.0<br>(78.8 to 118.5)     | 94.1<br>(56.5 to 151.0)     | 78.3<br>(32.3 to 96.4)      | 122.3<br>(99.6 to 163.9)    | 96.8<br>(60.1 to 118.3)     | 96.2<br>(60.0 to 151.8)    | 106.7<br>(54.7 to 112.9)  |
| Mean peak load (N) (range)             | 121.3<br>(39.5 to 200.0)    | 152.8<br>(129.8 to 242.3)   | 129.8<br>(72.8 to 229.4)    | 46.4<br>(26.1 to 62.8)      | 116.8<br>(76.4 to 145.6)    | 149.2<br>(56.8 to 176.3)   | 134.6<br>(106.3 to 220.0) |
| <b>Left control tibiae</b>             |                             |                             |                             |                             |                             |                            |                           |
| Mean BMC (mg) (range)                  | 27.3<br>(25.86 to 30.1)     | 27.2<br>(24.38 to 30.58)    | 27.4<br>(26.01 to 30.86)    | 29.2<br>(26.25 to 32.74)    | 27.3<br>(25.62 to 29.41)    | 28.1<br>(25.6 to 32.04)    | 28.1<br>(25.72 to 30.56)  |
| Mean BMD (mg/cm <sup>3</sup> ) (range) | 954.2<br>(873.7 to 1024.1)  | 975.2<br>(879.1 to 1018.9)  | 978.7<br>(889.2 to 1092.2)  | 1013.1<br>(969.7 to 1052.5) | 910.5<br>(763.6 to 1016.8)  | 944.2<br>(881.7 to 1071.8) | 988.5<br>(889.1 to 991)   |
| Mean CSA (mm <sup>2</sup> ) (range)    | 28.6<br>(25.47 to 30.47)    | 27.9<br>(24.32 to 30.4)     | 28.0<br>(26.25 to 29.25)    | 28.8<br>(26.09 to 31.65)    | 30.4<br>(26.6 to 38.52)     | 29.8<br>(26.78 to 30.04)   | 28.5<br>(26.9 to 34.37)   |
| Mean peak load (N) (range)             | 180.4<br>(165.11 to 210.74) | 177.8<br>(143.23 to 214.23) | 184.2<br>(162.42 to 220.94) | 40.9<br>(34.69 to 44.84)    | 194.5<br>(180.14 to 246.72) | 189.6<br>(185.24 to 244.7) | 200<br>(16.3 to 240.27)   |

\* BMC, bone mineral content; BMD, bone mineral density; CSA, cross-sectional area

consolidation period of four weeks, all the animals were killed by an intravenous injection of pentobarbital sodium (150 mg/kg), and both tibiae were stripped of soft tissue.

**Radiography and quantitative CT.** For radiography, both tibiae were orientated in anterior to posterior projections, with exposures obtained by TOREX Type 100P equipment (Toshiba Medical Supply Co. Ltd, Tokyo, Japan) using digital luminescent cassettes with a 60 kV and 4 mA exposure and processed using an AC-3CS developer (Fujix Co Ltd, Tokyo, Japan). The radiographs were examined for the presence of radiodense lines which have been observed in clinical and animal studies on N-BP treatment.<sup>12,22</sup> These lines, which were taken to demonstrate a systemic effect of the treatment, were counted by HO in proximal tibiae blindly by screening ID numbers on X-P (Fig. 2).

For quantitative CT (QCT) both tibiae were analysed using an XCT Research SA+ (Stratec Medizintechnik GmbH, Pforzheim, Germany) QCT scanner and analysis software (Rev.5.50e; Stratec Medizintechnik GmbH). A slice was obtained at the middle of the distracted bone and also from the left tibia 5 mm distal to the tibiofibular junction in the same line as the osteotomy of the right tibia, and used as a control. The software allowed the calculation of data on the BMD (mg/cm<sup>3</sup>), the bone mineral content (mg) and the cross-sectional area (mm<sup>2</sup>).

**Three-point bend destructive testing.** After QCT studies, the bones were frozen in saline-soaked gauze at -20°C and thawed for the bending test. Testing to failure was carried out by three-point bending in accordance with the American Society for Testing and Materials standard D 790-03.<sup>23</sup> The tibiae were placed in a mechanical testing machine (model 4465; Instron, Canton, Massachusetts) orientated with the posterior surface placed superiorly, with a support span of 80 mm. A bending force was applied to the distracted area at a displacement rate of 2 mm per minute using a 5 kN load cell. Data for load and displacement were

recorded on an accompanying computer every 200 ms until failure occurred. The breaking force was defined as the bending load at the moment of failure.

**Statistical analysis.** The mean results for each of the groups was compared using analysis of variance (ANOVA) followed by Tukey-Kramer's test for multiple comparisons. Statistical significance was set at  $p \leq 0.05$ .

## Results

Radiographs showed that all 47 distracted tibiae had fully united without osteomyelitis. The injection of alendronate did not cause any life-threatening complications.

**Radiography and quantitative CTs.** The results of the bone mineral content, BMD and cross-sectional area for each of the groups are presented in Table II. In the right distracted tibiae, QCT (Fig. 3) showed that in the local-saline group there was no significant effect on the mean bone mineral content, BMD or cross-sectional area compared with the systemic-saline group (ANOVA). The local-low group showed a mean increase in the BMD of 124.3 mg/cm<sup>3</sup> over the local saline group (ANOVA,  $p < 0.05$ ), while the local-mid group had a less effect which did reach statistical significance (ANOVA) (Table II). The local-mid group mean bone mineral content and mean cross-sectional area increased more than in the other local groups, but there were no significant differences (ANOVA). In the local groups, the mean bone mineral content increased in a dose-dependent manner. In the local-low group all the parameters increased more in the systemic-low group, but did not reach statistical significance (ANOVA) more than in the systemic-low group, and similarly in the local-mid group compared with the systemic-mid group. The systemic-high group had the highest values of all the groups, and the mean bone mineral content in this group was significantly higher than that in the systemic saline group (ANOVA,  $p < 0.05$ ), the systemic-mid group (ANOVA,  $p < 0.01$ ) and the

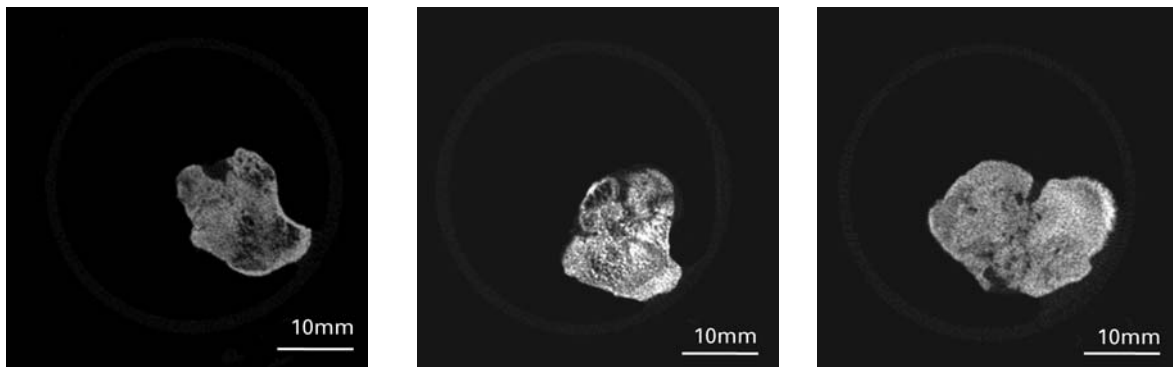


Fig. 3a

Fig. 3b

Fig. 3c

Quantitative CT images at the mid-point of the distracted callus area in a) the local-saline, b) the local-low and c) the local-mid groups.

**Table III.** The appearance ratios\* of radiodense lines in all groups

|              | Systemic-saline | Systemic-low | Systemic-mid | Systemic-high | Local-saline | Local-low | Local-mid |
|--------------|-----------------|--------------|--------------|---------------|--------------|-----------|-----------|
| Right tibiae | 0/7             | 4/7          | 6/7          | 5/5           | 0/7          | 3/7       | 6/7       |
| Left tibiae  | 0/7             | 0/7          | 0/7          | 2/5           | 0/7          | 0/7       | 0/7       |

\* the number of tibiae showing any radiodense lines in each group

local-saline group (ANOVA,  $p < 0.01$ ). The appearance ratio of radiodense lines<sup>12,22</sup> examined on radiographs increased in a dose-dependent manner in both the systemic and local groups. There were no radiodense lines identified in any of the tibiae of rabbits which had received systemic-saline and local-low treatment. All the right-sided tibiae in the systemic-high group had radiodense lines (Table III).

In the left-sided tibiae, the mean BMD of all groups increased in a dose-dependent manner. The mean bone mineral content and the mean cross-sectional area did not correlate with the dose of alendronate (Table II). However, there was no significant difference in the QCT scan results for the various groups (ANOVA). In the systemic-high group two of the five tibiae showed radiodense lines (Fig. 2) which were not evident in the other six groups (Table III).

**Mechanical strength.** All the tibiae which had undergone distraction fractured through the distracted area on the three-point bending test (Table II). The local-low group showed the highest increase in peak load of the local groups, and showed a mean increase in the peak load of 32.4 N over the local-saline group, but this did not reach statistical significance (ANOVA). In both the systemic and local groups, there was no dose-dependent increase in the peak load. The systemic-high group had a markedly decreased mean peak load to failure which was 74.9 N lower than that of the systemic-saline group (ANOVA) and significantly lower than that of the systemic-low group (ANOVA,  $p < 0.01$ ), the local-low group (ANOVA,  $p < 0.01$ ), the systemic-mid group (ANOVA,  $p < 0.05$ ), and the systemic-low group (ANOVA,  $p < 0.05$ ).

In the left-sided control tibiae, the three-point bending test showed that there was no dose-dependent relation for

the peak load. The systemic-high group had a significantly reduced peak load to failure compared with the other six groups (ANOVA,  $p < 0.01$ ).

**Discussion**

In the local-low group the BMD increased significantly more than in the local-saline group without producing radiodense lines or a reduction in bone strength. This finding supports the effectiveness of local administration of alendronate as also does the finding in the local-low and local-mid groups that all parameters in the QCT analysis increased more than those of the systemic-low and systemic-mid groups, respectively. The explanation might be that the injected alendronate was received directly on the local bone surface where it was taken up by osteoclasts. Systemically administered N-BP has been shown to increase the bone mineral content or BMD in a dose-dependent manner by suppression of osteoclasts during distraction osteogenesis.<sup>13,14</sup> However, our study showed that the BMD was increased in the local-low group more than in the local-mid group which agreed with the report that locally administered low-dose pamidronate increased BMD more than a high dosage in a rat fracture model.<sup>20</sup> Recent *in vitro* studies have shown that N-BP in low concentrations stimulates the differentiation of osteoblasts while it inhibits this differentiation at high concentrations.<sup>24-27</sup> It is presumed that in the local-mid group there was a negative effect on the activity of osteoblasts.

The strength of the distracted bone is an important consideration for the clinical application of locally administered bisphosphonate. The local-low group showed a mean increase in the peak load to failure of 32.4 N over the local-



saline group but this did not reach statistical significance. This result is in agreement with that of a previous report in which local pamidronate did not show any definite advantages as regards strength in a rat fracture model.<sup>20</sup> We were unable to show any mechanical benefit of the local-low treatment, and a further study is required using a larger number of rabbits to resolve this.

Initially the systemic-high treatment markedly decreased the peak load of the right tibiae while it increased the bone mineral content, BMD and cross-sectional area. This result contradicts the report from Little et al<sup>14</sup> who found that in a rabbit model high-dose N-BPs raised the strength by increasing bone volume in a dose-dependent manner during distraction osteogenesis. This difference may be a result of the different dosing regimes. In our study injections were made five times during five weeks while in the study by Little et al<sup>14</sup> injections were given twice during two weeks, based on the experience of Yaffe et al<sup>17</sup> who showed that the local level of alendronate decreased in a short period. Long-term suppression of bone remodelling by high-dose alendronate is considered to impair the intrinsic material properties of both the distracted right and control left tibiae.

The low- and mid-dosage groups did not show any radiodense lines on the left-sided control tibiae. Such lines were seen in the left control tibiae only in the systemic-high group, suggesting that the low or mid doses did not have detectable systemic effects. The local-low dose in our study equated approximately to 0.01 mg in a child weighing 20 kg. This dose was considered to be safe in a clinical report in which a dosage of 1 mg/kg per day of oral alendronate administered for two years did not cause major side-effects in children with osteogenesis imperfecta.<sup>28</sup> It was striking that the distracted right tibiae in the local-low or local-mid groups had radiodense lines whereas the control left tibiae of these groups did not.

In conclusion, locally administered low-dose alendronate increased the mean BMD with no adverse affect on bone growth in a rabbit distraction osteogenesis model. This method of administration of alendronate may provide an effective pharmacological means of improving formation of bone in distraction osteogenesis.

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